ATTACHMENT A

The Available Science, Including New Information, Indicates That Higher TI Levels For Both Parenteral And Enteral Exposure Likely Would Be Fully Protective Of Patient Health

American Chemistry Council Phthalate Esters Panel
December 4, 2002

The basis for CDRH's Draft Guidance on DEHP[1] is the safety assessment FDA published in September 2001.[2] That assessment found that, for some medical procedures, there was the possibility that exposure to DEHP could exceed the Tolerable Intake (TI) level established in the Safety Assessment. The parenteral TI was based on an intravenous study in neonatal rats, with a NOAEL of 60 mg/kg/day and a LOAEL of 300 mg/kg/day (AdvaMed 2001).[3] The enteral TI was based on an oral study in rats with a NOAEL of 4 mg/kg/day and a LOAEL of 38 mg/kg/day (Poon et al. (1997).[4] In both cases, the endpoint of concern was testicular effects. The TIs were set at levels 100-fold below the respective NOAELs.

Although the Safety Assessment found that some estimated exposures exceeded these TIs, the Panel believes the information in the Safety Assessment shows the risk of potential health effects to be low for the following reasons:

- In most cases, average or median exposure levels did not exceed the TI.
- In most cases, where the upper-bound estimated exposure exceeded the TI, it was by only a small amount. The TI is 100-fold below the NOAEL. Thus, the upper-bound exposure is still well below a level at which no effects were observed in the animal studies.
- Even the highest exposure estimated by FDA – for a neonatal infant receiving multiple intensive procedures – was below the animal NOAEL.

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1 CDRH (2002). *Medical Devices Made with Polyvinylchloride (PVC) Using the Plasticizer di-(2-Ethylhexyl)phthalate (DEHP); Draft Guidance for Industry and FDA.* U.S. Food and Drug Administration, Center for Devices and Radiological Health, Rockville, MD.

2 CDRH (2001). *Safety Assessment of Di(2-ethylhexyl)phthalate (DEHP) Released from PVC Medical Devices.* U.S. Food and Drug Administration, Center for Devices and Radiological Health, Rockville, MD [hereafter "Safety Assessment"].

3 AdvaMed (2001). *21-day repeat dose male reproductive tract study of di(2-ethylhexyl)phthalate (DEHP) administered either intravenously or orally to rats starting at neonatal age 3-5 days, with satellite recovery group through 90 days of age.* Advanced Medical Technology Association (AdvaMed), Study number 11947, Washington, DC.

4 Poon R, Lecavalier P, Mueller R, Valli V, Procter B, and Chu I (1997). Subchronic oral toxicity of di-n-Octyl Phthalate and di(2-Ethylhexyl) Phthalate in the Rat. *Food Chem. Toxicol.* 35:225-239.

- In most cases, the exceedances involved estimated exposures for medical procedures that would be performed in a day or over a few days. However, the studies that were the basis for the TIs involved exposures for several weeks.

- Some of the exceedances were for medical procedures in adults, who are past the developmental stage, whereas the concern for testicular effects is primarily for developing organisms.

Furthermore, the scientific data indicate that the TIs used by FDA are very conservative, and that much higher TIs would be protective of human health. This is underscored by data that have recently become available and that are discussed below.

New Data Demonstrate that Even Developing Primates Are Much Less Susceptible to DEHP than Are Rodents

As FDA was aware when it conducted its safety assessment, studies in primates have demonstrated that they are less susceptible to the effects of DEHP than are rodents. *No* testicular histopathology has been observed in preadolescent primates exposed to doses of 500 mg/kg/day or in adult primates at 2,500 mg/kg/day -- levels which would produce effects in rodents.[5] While this data provided a strong indication that primates are indeed less sensitive than rodents, it did not fully address potential reproductive toxicity tract effects in primates exposed to DEHP early in life. That issue has now been addressed by a study recently conducted in Japan.

In the Japanese study, marmosets (5 animals per dose group) were dosed with 100, 500 and 2500 mg/kg/day of DEHP from weaning through sexual maturation. The study scientists have informed the Panel that there were no effects of DEHP on any target organ (including the testes), there was no substantial accumulation of DEHP or its metabolites in the testes, and there was no gross or microscopic evidence of testicular changes at any dose. The study scientists therefore conclude that DEHP, at doses up to 2500 mg/kg/day, does not affect the maturation of the primate testis. The Panel will provide additional information on this study as it becomes available.

This study provides further evidence that even developing primates are far less sensitive to the effects of DEHP than are rodents. Since non-human primates are a much better model for humans than are rodents,[6] these data indicate that humans are not likely to experience

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5 Kurata Y, Kidachi F, Yokoyama M, Toyota N, Tsuchitani M, and Katoh M (1998). Subchronic toxicity of di(2-ethylhexyl) phthalate in common marmosets: Lack of peroxisomal proliferation, testicular atrophy, or pancreatic acinar cell hyperplasia. *Toxicological Sciences* 42:49-56; Pugh G, Isenberg J, Kamendulis L, Ackley D, Clare L, Brown R, Lington A, Smith J, and Klaunig J (2000). Effects of di-isononyl phthalate, di-2-ethylhexyl phthalate, and clofibrate in Cynomolgus monkeys. *Toxicological Sciences* 56:181-188.

6 *See, e.g.,* Sharpe R, Walker M, Millar M, Atanassova N, Morris K, McKinnell C, Saunders P, and Fraser H (2000). Effect of neonatal gonatotropin-releasing hormone antagonist administration on Sertoli cell number and testicular development in the marmoset: comparison with the rat. *Biol. Reprod.* 62:1685-1693.

effects at a level that causes no effects in rodents, much less at the TI levels that are set 100-fold below the rodent NOAELs.

Data Published After Poon et al. (1997) Indicate that the NOAEL for Testicular Pathology, Even for Pre-Natal Exposures, Is Approximately 100 mg/kg/day

FDA's enteral TI was based on Poon et al. (1997), which reported that subchronic administration of DEHP to juvenile male rats resulted in seminiferous tubule atrophy and Sertoli cell vacuolation; the no-observed-effect level for Sertoli cell vacuolation was judged to be 50 ppm in the diet, or approximately 3.7 mg/kg/day. However, several other recent studies have not replicated these findings, and indicate that the true oral NOAEL for testicular pathology is about 100 mg/kg/day.

Schilling *et al.* (2001)[7] administered DEHP to groups of twenty-five male and female Wistar rats, with subsequent mating for two generations. The exposure was dietary at 1000, 3000, and 9000 ppm (approximately 113, 340 and 1088 mg/kg/day), and was continuous from at least 70 days premating of the first parental generation to sacrifice (i.e., exposure was from conception to sacrifice for the two offspring generations). The study evaluated a number of reproductive and developmental toxicity parameters.[8]

At 1088 mg/kg/day, the rats experienced reduced body weight gain, increased liver weight, reduced fertility, adverse effects on sperm and testes, and reduced survival and growth of offspring. At 340 mg/kg/day, the rats had reduced survival rates and growth of their offspring, and effects on indicators of reproductive development. At 113 mg/kg/day, there were no effects other than peroxisomal proliferation – an effect to which rodents are known to be susceptible but to which humans are refractory. Based on this assessment, the authors reported that the NOAEL for developmental toxicity (survival, growth and development of offspring) was 113 mg/kg body weight/day.

Schilling *et al.* also assessed the effects on the nervous system of exposure to DEHP in a subset of the second generation of rat offspring. Tests included motor activity tests, a water maze test, and functional observations. The authors found no substance-induced impairment of neurofunction at any dose and concluded that the NOAEL for nervous system effects was greater than 1000 mg/kg/day.

As part of the overall evaluation of reproductive toxicity, testes were taken from parental males, fixed in Bouin's solution, and examined microscopically. Of particular importance is the examination of testes from males from the first filial (F1) generation since these animals were exposed to DEHP continuously from conception to terminal sacrifice. Mild

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7 Schilling K, Gembardt C, and Hellwig J (2001). *Di-2-ethylhexyl phthalate – two-generation reproduction toxicity study in Wistar rats. Continuous dietary administration.* Experimental Toxicology and Ecology, BASF Aktiengesellschaft, D-67056 Ludwigshafen, FRG. Laboratory project identification 70R0491/97139.

8 The parameters included food consumption, body weight, fertility, viability, birth weight, organ weights, pathology, estrous cyclicity, sperm parameters, anogenital distance, preputial separation, vaginal opening, areolae, and ovarian examination.

seminiferous tubular atrophy was reported at 340 mg/kg/day, consistent with Poon et al., who reported minimal to mild tubular atrophy at approximately 370 mg/kg/day, but no such effects at 37 mg/kg/day. Unlike the Poon study, however, there was no evidence of Sertoli cell vacuolation in otherwise normal tubules in the Schilling study. The overall NOAEL for pathological changes in the testes was 113 mg/kg/day. As the Schilling et al. study involved both gestational and neonatal exposure and used the preferred Bouin's fixative, its results should be carefully considered in relation to Poon *et al.* in determining the accurate NOAEL. BASF Corporation separately is submitting a copy of the Schilling study to FDA.

Dalgaard et al. (2000)[9] investigated the testicular effects of DEHP on juvenile Wistar rats. Testicular atrophy was found in animals exposed to 5000 or 10,000 mg/kg/day, but there were no pathological changes in animals exposed to 1000 mg/kg/day.

Akingbemi et al. (2001)[10] investigated the effects of DEHP administered either *in utero* or to prepubertal Long-Evans rats. Although effects on testosterone levels were obtained when the DEHP was given during *in utero* development, there were no effects on testicular weight and no pathological changes at levels up to 200 mg/kg/day.[11]

Wolfe et al. (2002)[12] conducted a study in Sprague-Dawley rats, using a continuous-breeding protocol (that is, including gestational exposure). No treatment-related effects, including no microscopic findings in the testes, were observed at levels of 1000 ppm (approximately 100 mg/kg/day). The results of this study were presented at the Society of Toxicology (SOT) meeting on March 20, 2002, and release of the full report is anticipated in the near future. A copy of the SOT abstract is provided with these comments.

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9 Dalgaard M, Ostergaard G, Lam H, Hansen E, and Ladefoged O (2000). Toxicity study of di(2-ethylhexyl)phthalate (DEHP) in combination with acetone in rats. *Pharmacology and Toxicology* 86:92-100.

10 Akingbemi B, Youker R, Sottas C, Ge R, Katz E, Klinefelter G, Zirkin G, and Hardy M. (2001). Modulation of rat leydig cell steroidogenic function by di(2-ethylhexyl) phthalate. *Biology of Reproduction* 65:1252-1259.

11 The Panel notes that the effects on testosterone are not themselves a proper endpoint for risk assessment. As stated by Moore et al. (1995): "Various [biochemical] markers of [reproductive] exposure and effect have been investigated in male reproductive toxicology, including ... androgens ... currently, however, they cannot be considered evidence of male reproductive toxicity." Moore M, Daston G, Faustman E, Golub M, Hart W, Hughes C, Kimmel C, Lamb J, Schwetz B, and Scialli A (1995). An evaluative process for assessing human reproductive and developmental toxicity of agents. *Reproductive Toxicology* 9(1):61-95.

Similarly, in its Safety Assessment of DEHP, FDA stated: ""Only studies with effects broadly considered to be adverse (histopathological or functional changes) will serve as the basis for T1 derivation." Akingbemi et al. identified a "subclinical" or "precursor" effect, but this does not seem to be associated with either pathological or functional changes, as shown by their own data, and corroborated by Dalgaard et al. (2000) and Schilling et al. (2001).

12 Wolfe G, Layton K, Nehrebeckyi L, Wang Y, Chapin R, Rouselle S, and Bishop J (2002). Reproductive effects of diethylhexylphthalate (DEHP) in Sprague-Dawley rats when assessed by the continuous breeding protocol. *The Toxicologist* 66(1-S):abstract 1147.

Thus, in four separate studies, the Poon et al. finding of testicular pathology at a relatively low level has not been replicated. The basis for calling 3.7 mg/kg/day a NOAEL in the Poon et al. study was the observation of Sertoli cell vacuolation – characterized by the authors as "mild" – at 38 mg/kg/day. Based on the data from the foregoing three studies, the Panel believes that the relevance of the vacuoles for risk assessment is questionable. None of these studies showed evidence of vacuolation in the testes of animals treated perinatally with DEHP. Furthermore, work by Akingbemi et al. (2001), AdvaMed (2001), Dostal et al. (1988),[13] and Kuwada et al. (2002)[14] indicate that adverse effects observed in neonatal animals immediately after exposure are reversible and that no difference between treated and control animals is evident for testes histopathology, sperm production, or fertility when the animals are mature. Thus, it seems inappropriate to establish acceptable exposure levels based on a transient effect in prepubescent animals that has no impact in the mature animal (the ultimate subject for concern). The Panel therefore believes it is inappropriate to continue to use the NOAEL derived from Poon et al. as the basis for assessments of DEHP. The data indicate that the appropriate testicular pathology NOAEL for oral exposures is approximately 100 mg/kg/day.[15]

The Data Indicate that the Parenteral NOAEL of 60 mg/kg/day Is Very Conservative

FDA's parenteral TI was based on the NOAEL of 60 mg/kg/day in the AdvaMed (2001) rat study. However, the data indicate that this is a very conservative NOAEL:

- The LOAEL in the AdvaMed study was 300 mg/kg/day. While reproductive tract effects were seen at that level, they apparently were not pronounced, and all observed effects were reversible, except for testicular weight.[16] Thus, the "true" NOAEL for DEHP may be nearer to 300 mg/kg/day than to 60 mg/kg/day.

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13 Dostal L, Chapin R, Stefanski S, Harris M, Schwetz B. (1988). Testicular toxicity and reduced Sertoli cell numbers in neonatal rats by di(2-ethylhexyl)phthalate and the recovery of fertility as adults. *Toxicol Appl Pharmacol* 95:104-121.

14 Kuwada M, Kawashima R, Nakamura K, Kijima H, Hasumi H, Maki J, and Sugano S (2002). Neonatal exposure to endocrine disruptors suppresses juvenile testis weight and steroidogenesis but spermatogenesis is considerably restored during puberty. *Biochemical and Biophysical Research Communications* 295:193-197.

15 This NOAEL also is consistent with effect levels for other endpoints in oral rodent studies. For example, the overall LOAEL in a recent chronic study in rats was 147 mg/kg/day, and the LOAEL in a chronic study of mice was 292 mg/kg/day. David R, Moore M, Finney D, and Guest D (2000). Chronic toxicity of di(2-ethylhexyl)phthalate in rats. *Toxicological Sciences* 55:433-443; David R, Moore M, Finney D and Guest D (2000). Chronic toxicity of di(2-ethylhexyl)phthalate in mice. *Toxicological Sciences* 58:377-385; David R, Moore M, Finney D, and Guest D (2001). Reversibility of the Chronic Effects of Di(2-ethylhexyl)phthalate. *Toxicol. Pathol.* 29:430-439.

16 At 21 days, effects seen in the 300 mg/kg/day dose group were testicular atrophy, depletion of seminiferous tubules, and depletion of germ cells. After the recovery period, none of the histopathological changes were observed and no effects were seen in a functional assessment of male reproductive capacity (sperm count, motility and morphology).

- As discussed above, the appropriate rat oral NOAEL for testicular effects is about 100 mg/kg/day. As FDA has acknowledged, DEHP exhibits lower toxicity by the intravenous route than by the oral route. Therefore, it is likely that the parenteral NOAEL is greater than 100 mg/kg/day.

- Sjoberg et al. (1985) identified a NOAEL of 25 mg/kg/day and a LOAEL of 250 mg/kg/day for testicular effects in rats receiving intravenous doses of DEHP. Baxter (2000) found no histopathological changes in the testes of neonatal rats and rabbits exposed intravenously to DEHP at 62 mg/kg/day. Both of these studies are consistent with an intravenous NOAEL greater than 100 mg/kg/day.

- There are some human data that address the question of whether humans might be susceptible to developmental effects due to parenteral exposure to DEHP. Survey data from pregnant dialysis patients have found no developmental toxicity associated with exposure to DEHP.[17]

Based on the foregoing, it appears likely that the actual parenteral rat NOAEL is greater than 100 mg/kg/day. Furthermore, based on the recent Japanese study in marmosets and the information on pregnant dialysis patients, it is likely that a TI based on the parenteral rat NOAEL is very conservative.

The Data Indicate that Higher TIs Would Be Protective of Human Health and that Risks to Patients from DEHP-Containing Devices Are Very Low

The Panel believes that the science discussed above supports establishment of TI values that are higher than those CDRH developed in its Safety Assessment. Thus, even where the Safety Assessment found the possibility of exceedances of the TI, the likelihood of a significant risk to patients is very low.

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17 Reister F, Reister B, Heyl W, Riehl J, Schroder W, Mann W, and Rath W (1999). Dialysis and pregnancy -- A case report and review of the literature. *Renal failure* 21:533-539; Toma H, Tanabe K, Tokumoto T, Kobayashi C, and Yagisawa T (1999). Pregnancy in women receiving renal dialysis or transplantation in Japan: a nationwide survey. *Nephrol. Dial. Transplant.* 14:1511-1516; Chan W, Okun N, Kjellstran C (1998). Pregnancy in chronic dialysis: a review and analysis of the literature. *Artificial Kidney and Dialysis* 21:259-268; Blowey D, and Warady B (1998). Neonatal outcome in pregnancies associated with renal replacement therapy. *Advances in Renal Replacement Therapy* 5:45-52.

Session: Developmental Toxicity and Teratology II

Final ID: 1147 **Day / Time:** Wednesday, Mar. 20, 9:30 AM - 12:30 PM

Location: Exhibit Hall

REPRODUCTIVE EFFECTS OF DIETHYLHEXYLPHTHALATE(DEHP) IN SPRAGUE-DAWLEY RATS WHEN ASSESSED BY THE CONTINUOUS BREEDING PROTOCOL.

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DEHP was evaluated using a multi-generational protocol in rats to assesspotential reproductive effects over multiple generations. Beginning on Study Day 1, DEHP was administered *via* the diet at doses of 1.5 (control), 10, 30, 100, 300, 1000, 7500, and 10, 000 ppm to adult male and female rats (N=17). Each mating generation was allowed to produce three litters (F_{1a}, 1b, 1c, F_{2a}, 2b, 2c, F_{3a}, 3b, 3c). On PND 81 $\pm$ 10, animals from the third litter from each generation were assigned to mating pairs (N=17). Reductions were noted in mean body weight of animals receiving 7500 or 10, 000 ppm. Reproductive/litter effects including decreases in pregnancy indices for most generations; decreases in the no. of males per litter and the total no. of pups per litter; decreases in male and female pup weights at birth and during lactation; decreases in anogenital distance for male pups; and delay of sexual development parameters were noted at the top 2 dose groups. The 10, 000 ppm animals did not produce any F_2 generation animals. Outbreedings at 7500 and 10, 000 ppm of treated males with naive females revealed decreases in mating, pregnancy and fertility indices and decreases in the number of implantation sites. Sperm end-points were decreased at 7500 ppm in all generations and at 10, 000 ppm in the F_0 and F_1 generations. Organ weight changes of the liver (increase), kidney (increase), and male accessory sex organs (decrease) occurred at 1, 000, 7500, and 10, 000 ppm. Treatment-related microscopic findings were noted at 7500 and 10, 000 ppm in the testes, epididymides, liver, adrenal, and kidney in all generations and in the liver at 1, 000 ppm in the F_1 and F_2 generations. DEHP is considered a reproductive toxicant at 7500 and 10, 000 ppm. There was no general or reproductive effects observed at dose levels at or below 1, 000 ppm except liver toxicity at 1, 000 ppm.